In addition to the framework changes at selected amino acid positions, the CDRs also are altered to contain a plurality of different amino acid residue changes at all or selected positions within the donor CDRs. For example, random or biased incorporation of the twenty naturally occurring amino acid residues, or preselected subsets, are also introduced into the donor CDRs to produce a diverse population of CDR species. Including a diverse population of different CDR variant species ensures that beneficial changes in the framework positions are not neutralized by a conformationally incompatible residue in a donor CDR. Inclusion of CDR variant species into the diverse population of variable regions also allows for the generation of variant species that exhibit optimized binding affinity for a predetermined antigen.

The resultant population of CDR grafted variable regions described above will therefore contain, at the relevant framework positions and at the selected CDR positions, a species corresponding to the authentic parent amino acid residue at each position as well as a diverse number of different species which correspond to the possible combinations and permutations of the authentic parent amino acid residues together with the variant residues at each of the relevant framework and selected CDR positions. Such a diverse population of CDR grafted variable regions are screened for an altered variable region species which retains donor CDR binding activity, or optimized binding activity.

One advantage of the methods of the invention is that they do not limit the choice of acceptor variable regions applicable, or expected to be successful, for receiving CDRs from the donor molecule. For example,

when choosing an acceptor region it can be desirable, or in some circumstances even required, to select an acceptor that is closely similar to the variable region amino acid sequence harboring the donor CDRs because the CDR conformation in the grafted variable region will likely be more similar to that of the donor. However, selecting similar framework region sequences between the donor and acceptor variable regions still does not provide which residues, out of the differences, actually play a role in CDR binding affinity of the grafted variable region. Selection of similar acceptor frameworks therefore only limits the number of possible residues which to investigate in order to reacquire binding affinity onto the grafted variable region. The methods of the invention circumvent this problem by producing a library of all possible or relevant changes in the acceptor framework, and then screening those variable regions, or heteromeric binding fragments thereof for species that maintain or exhibit increased binging affinity compared to the donor molecule. Therefore, the applicability is not preconditioned on the availability or search for an acceptor framework variable region similar to that of the donor.

Selection of the relevant framework amino acid positions to alter can depend on a variety of criteria well known to those skilled it the art. As described above, one criteria for selecting relevant framework amino acids to change can be the relative differences in amino acid framework residues between the donor and acceptor molecules. Selection of relevant framework positions to alter using this approach is simple and has the advantage of avoiding any subjective bias in residue determination or any inherent bias in CDR binding affinity contribution by the residue. Criteria other

than relatedness of amino acid residues can be used for selecting relevant framework positions to alter. Such criteria can be used in combination with, or alternative to the selection of framework positions having divergent amino acid residues. These additional criteria are described further and similarly are well known to those skilled in the art.

Another criteria which can be used for determining the relevant amino acid positions to change can be, for example, selection of framework residues that are known to be important, or contribute to CDR conformation. For example, canonical framework residues play such a role in CDR conformation or structure. Such residues can be considered to be relevant to change for a variety of reasons, including for example, their new context of being associated with heterologous CDR sequences in the grafted variable region. Targeting of a canonical framework residue as a relevant position to change can identify a more compatible amino acid residue in context with its associated donor CDR sequence. Additionally, targeting of canonical residues can allow for the identification of residues at these positions that absorb detrimental effects to CDR structure from residues located elsewhere in the framework region.

The frequency of an amino acid residue at a particular framework position is another criteria which can be used for selecting relevant framework amino acid positions to change. For example, comparison of the selected framework with other framework sequences within its subfamily can reveal residues that occur at minor frequences at a particular position or positions. Such positions harboring less abundant residues are similarly